A good baking-quality nonfat dry milk (NFDM) was fractionated by isoelectric precipitation. The soluble protein fraction (whey) was responsible for the loaf volume increase obtained with NFDM. The soluble fraction was dialyzed to separate the solub material retained in the bag) and solub (material passing through the bag) fractions. Both fractions contributed to loaf volume. When the solub fraction was fractionated further by ion-exchange chromatography, the anion fraction alone was equal to NFDM. The ammonium ion used to elute the anion fraction was apparently responsible for the loaf volume increase of that fraction. Ammonium ion, in the form of diammonium phosphate, functionally replaced NFDM in breadmaking.

Nonfat dry milk (NFDM) has traditionally been a common ingredient in bread dough. Effects of milk in dough have been reviewed by Brouitt et al. (1) and Kinsella (2). However, the biochemical role of NFDM in improving or depressing bread quality is not completely understood. Although the loaf-volume depressing effect of raw milk has been studied extensively (3–7) and the beneficial effects of heat treatment are well documented (8,9), the beneficial functional effects of heat-treated NFDM in bread have received little attention.

St. John and Bailey (10) demonstrated buffering action of NFDM in fermenting dough and showed that doughs containing NFDM produced more carbon dioxide than regular doughs. Bohn and Bailey (11) observed that doughs containing NFDM were easier to mold and more pliable than nonmilk doughs. Nonfat dry milk in doughs increased the tolerance of flour to potassium bromate and partially prevented deleterious effects of excess potassium bromate on loaf volume, crumb grain, and texture (12,13). Doughs containing NFDM and optimum potassium bromate gave bread with higher volumes and better texture than did doughs containing optimum potassium bromate but no milk. Eisenberg (14) pointed out that shortening was necessary to obtain the increased response with potassium bromate.

Recently, the use of NFDM in breadmaking has declined. Several reasons appear to be important in this decline: 1) the increased price of NFDM, which makes it less attractive to the baker; 2) the increased use of short-time or continuous baking systems where NFDM appears to be at least somewhat detrimental; and 3) the shortage of good baking-quality NFDM on the market.

This study was undertaken to identify the fraction(s) of a good baking-quality heat-treated NFDM responsible for the improved loaf volume of doughs containing NFDM.
MATERIALS AND METHODS

One hundred grams of commercially available NFDM (Galloway West, Fond-du-Lac, Wis.) was suspended in 1500 ml of distilled water at room temperature. The pH of the suspension was 6.6–6.7. The milk was fractionated (Fig. 1) by isoelectric precipitation at pH 4.5 using lactic acid (1:3, acid:H₂O). The pH 4.5 insoluble components (insol), mainly caseins, were recovered by centrifugation and washed twice with distilled water. The supernatants (milk whey) were combined, and dialyzed against distilled water for at least 36 hr in the cold (4°C). The dialyzable components were combined and the pH adjusted to 6.6 with 0.5N sodium carbonate. All fractions were dried by lyophilization.

Protein and moisture were determined by standard procedures (15). The baking formula included: flour, 100 g; sugar, 6 g; salt, 1.5 g; malt, 0.75 g; shortening, 3.0 g; NFDM (if any), 4.0 g; and yeast, 2.0 g. A straight-dough method was used with optimum mixing time, absorption, and potassium bromate. All dry milk components were mixed with the flour thoroughly before any other baking ingredients were added. Doughs were fermented 3 hr and proofed 55 min at 30°C, humidity 86%. Punching and molding were mechanical. Baking time was 25 min at 218°C. Loaf volume was measured by rapeseed displacement within 3 min after loaves came from the oven. At least duplicate loaves were baked and the standard deviation was 12 cc.

Flour used was an untreated, commercial, hard winter wheat flour containing 11.9% protein and 0.40% ash.

RESULTS AND DISCUSSION

Fractionation and Reconstitution of NFDM

Fractionation of NFDM. A baker's grade of NFDM was fractionated by the scheme shown in Fig. 1. The insol, sol, sol₀, and so₁₀₂ fractions were lyophilized to give dry hygroscopic powders. The percentage of each fraction obtained is given in Table 1.

![Fractionation scheme of nonfat dry milk](image)

Fig. 1. Fractionation scheme of nonfat dry milk.
The insoluble fraction was a creamy-white solid assumed to be mainly caseins. The insoluble fraction probably also included some of the whey proteins (lactoglobulin and lactalbumin) that had been denatured during the heating step in the manufacture of NFDM. Lactose was the major component of the soluble fraction with the remainder of the whey proteins, peptides, and inorganic materials. Greenishness of the soluble fraction was characteristic of riboflavin. The sol_\text{D} fraction was the material remaining in the dialysis tubing after dialysis for 36 hr at 4°C. The sol_\text{D} fraction was slightly opaque and contained the remainder of the whey protein. The sol_\text{HZ} fraction, the material passing through the dialysis bag, was a clear greenish-yellow and contained lactose, inorganic material, and low-molecular-weight organic material such as amino acids, peptides, and vitamins.

Baking Results with NFDM and its Fractions. Doughs containing 4% NFDM had a loaf volume 111 cc higher than those containing no NFDM (Table I). When the insoluble fraction alone was added to flour at its level in NFDM (36.1% of 4 g, or 1.444 g) and baked into bread, the loaf volume was 823 cc, only slightly better than the loaf volume with no added NFDM (812 cc). The soluble fraction (63.9% of 4 g, or 2.556 g) gave a loaf volume of 900 cc, nearly equal to the control loaf that contained 4% unfractionated NFDM (923 cc). Doughs containing both the insoluble and soluble fractions (completely reconstituted NFDM) gave a loaf volume nearly equal to the control (unfractionated NFDM), thus showing that the isoelectric fractionation had not altered the NFDM baking character.

From those results, it appeared that the factor responsible for increased loaf volume was in the soluble fraction of NFDM. The soluble fraction was fractionated further by dialysis. The sol_\text{D} fraction, added to flour at the appropriate level (22.3% of 4 g, or 0.892 g) and baked into bread, gave a loaf volume of 860 cc or about half the response of unfractionated NFDM. The sol_\text{HZ} fraction, similarly added to flour (39.9% of 4 g, or 1.596 g) and baked, gave a loaf volume of 885 cc or about 66% the response of NFDM. Thus, the factors

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Fraction Yield</th>
<th>Loaf Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFDM</td>
<td>100</td>
<td>923</td>
</tr>
<tr>
<td>No NFDM</td>
<td>...</td>
<td>812</td>
</tr>
<tr>
<td>Insoluble</td>
<td>36.1</td>
<td>823</td>
</tr>
<tr>
<td>Soluble</td>
<td>63.9</td>
<td>900</td>
</tr>
<tr>
<td>Insol + sol</td>
<td>...</td>
<td>900</td>
</tr>
<tr>
<td>Sol_\text{D}</td>
<td>22.3</td>
<td>860</td>
</tr>
<tr>
<td>Sol_\text{HZ}</td>
<td>39.9</td>
<td>885</td>
</tr>
<tr>
<td>Insol + sol_\text{D} + sol_\text{HZ}</td>
<td>...</td>
<td>905</td>
</tr>
<tr>
<td>Sol_\text{HZ} (A)</td>
<td>3.85</td>
<td>916</td>
</tr>
<tr>
<td>Sol_\text{HZ} (N + C)</td>
<td>36.05</td>
<td>809</td>
</tr>
<tr>
<td>Insol + sol_\text{D} + sol_\text{HZ} (A) + sol_\text{HZ} (N + C)</td>
<td>...</td>
<td>920</td>
</tr>
</tbody>
</table>
responsible for increasing loaf volume with NFDM appear to be in both the solD and solDZ fractions. Reconstituting the insol, solD, and solDZ fractions and baking them into bread gave a loaf volume nearly equal to that with unfracti

ON NFDM. Thus, the dialysis step did not alter NFDM's breadmaking character.

The solDZ fraction was fractionated further into an anion fraction and a cation plus neutral fraction by an anion ion-exchange column. The anion fraction was added to flour at the appropriate level (3.85% of 4 g, or 0.154 g) and baked into bread (Table I). The loaf volume was equivalent to the control with 4% NFDM. When added similarly, the cation plus neutral fraction gave a loaf volume equivalent to the control containing no NFDM. Reconstitution of the sol plus solD plus the two ion-exchange fractions and their addition to flour gave a loaf volume equivalent to the 4% unfracti

ON NFDM. Thus, fractionation with the anion column did not appear to alter the baking character of the NFDM fractions.

Data to this point indicate that the fraction retained on an anion column was the factor responsible for the increase in loaf volume obtained with 4% NFDM. However, it was not clear why the solDZ fraction did not give a loaf volume comparable to the control, because it contains the material found in the anion fraction.

In searching for an explanation of the somewhat confusing data, it occurred to us that elution of anionic material from the ion-exchange column with ammonium carbonate would give phosphate as diammonium phosphate. Therefore, diammonium phosphate (0.2% of flour weight) was added to flour and baked into bread. The loaf volume was fully comparable to the control loaf containing 4% NFDM. Baking with other ammonium salts gave similar results.

Fig. 2. Gas production for doughs containing no nonfat dry milk, 4% nonfat dry milk, and diammonium phosphate (0.2% based on flour weight).
The external and internal characteristics of bread baked with ammonium salts were also comparable to the control loaf containing 4% NFDM, except the crust color was slightly pale and the potassium bromate requirement was less. The baking formula used did not contain a yeast food. A common yeast food (16) contains 9.4% ammonium chloride (many others contain lower levels of ammonium compounds) and is normally used at 0.25 to 0.5% based on the flour weight. At the 0.5% level of usage, that yeast food would contribute 0.016% ammonium ion, or less than one-third the level of ammonium ion (0.054) in 0.2% diammonium phosphate.

Gassing power data showed that, after 4 hr of fermentation, doughs containing 4% NFDM had a higher rate of gas production than those without milk. However, dough containing diammonium phosphate had a higher rate of gas production than either of the others (Fig. 2).

Thus, the possible role of 4% NFDM in bread is to supply a source of ammonium ion for yeast.

Acknowledgment

This work was supported, in part, by a grant from Dairy Research, Inc.

Literature Cited


[Received September 12, 1975. Accepted January 23, 1976]